

# SEQUENCE LISTING



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JUL 08 2002

TECH CENTER 1600/2900

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Caldwell, Robert M.  
Baeck, Andre C.

<120> Multiply-Substituted Protease Variants

<130> GC502-2-C1

<140> US 10/033,325

<141> 2001-11-02

<150> US 09/178,155

<151> 1998-10-23

<150> US 08/956,323

<151> 1997-10-23

<150> US 08/956,564

<151> 1997-10-23

<150> US 08/956,324

<151> 1997-10-23

<160> 6

<170> FastSEQ for Windows Version 3.0

<210> 1

<211> 1497

<212> DNA

<213> Bacillus amyloliquefaciens

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<221> CDS

<222> (96)...(1245)

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Met Arg Gly Lys Lys Val	
1 5	
tgg atc agt ttg ctg ttt gct tta gcg tta atc ttt acg atg gcg ttc	161
Trp Ile Ser Leu Leu Phe Ala Leu Ala Leu Ile Phe Thr Met Ala Phe	
10 15 20	
ggc agc aca tcc tct gcc cag gcg gca ggg aaa tca aac ggg gaa aag	209
Gly Ser Thr Ser Ser Ala Gln Ala Ala Gly Lys Ser Asn Gly Glu Lys	
25 30 35	
aaa tat att gtc ggg ttt aaa cag aca atg agc acg atg agc gcc gct	257
Lys Tyr Ile Val Gly Phe Lys Gln Thr Met Ser Thr Met Ser Ala Ala	

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aag aag aaa gat gtc att tct gaa aaa ggc ggg aaa gtg caa aag caa Lys Lys Lys Asp Val Ile Ser Glu Lys Gly Gly Lys Val Gln Lys Gln 55 60 65 70			305
ttc aaa tat gta gac gca gct tca gtc aca tta aac gaa aaa gct gta Phe Lys Tyr Val Asp Ala Ala Ser Val Thr Leu Asn Glu Lys Ala Val 75 80 85			353
aaa gaa ttg aaa aaa gac ccg agc gtc gct tac gtt gaa gaa gat cac Lys Glu Leu Lys Lys Asp Pro Ser Val Ala Tyr Val Glu Glu Asp His 90 95 100			401
gta gca cat gcg tac gcg cag tcc gtg cct tac ggc gta tca caa att Val Ala His Ala Tyr Ala Gln Ser Val Pro Tyr Gly Val Ser Gln Ile 105 110 115			449
aaa gcc cct gct ctg cac tct caa ggc tac act gga tca aat gtt aaa Lys Ala Pro Ala Leu His Ser Gln Gly Tyr Thr Gly Ser Asn Val Lys 120 125 130			497
gta gcg gtt atc gac agc ggt atc gat tct tct cat cct gat tta aag Val Ala Val Ile Asp Ser Gly Ile Asp Ser Ser His Pro Asp Leu Lys 135 140 145 150			545
gta gca agc gga gcc agc atg gtt cct tct gaa aca aat cct ttc caa Val Ala Ser Gly Ala Ser Met Val Pro Ser Glu Thr Asn Pro Phe Gln 155 160 165			593
gac aac aac tct cac gga act cac gtt gcc ggc aca gtt gcg gct ctt Asp Asn Asn Ser His Gly Thr His Val Ala Gly Thr Val Ala Ala Leu 170 175 180			641
aat aac tca atc ggt gta tta ggc gtt gcg cca agc gca tca ctt tac Asn Asn Ser Ile Gly Val Leu Gly Val Ala Pro Ser Ala Ser Leu Tyr 185 190 195			689
gct gta aaa gtt ctc ggt gct gac ggt tcc ggc caa tac agc tgg atc Ala Val Lys Val Leu Gly Ala Asp Gly Ser Gly Gln Tyr Ser Trp Ile 200 205 210			737
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gat aaa gcc gtt gca tcc ggc gtc gta gtc gtt gcg gca gcc ggt aac Asp Lys Ala Val Ala Ser Gly Val Val Val Val Ala Ala Ala Gly Asn 250 255 260			881
gaa ggc act tcc ggc agc tca agc aca gtg ggc tac cct ggt aaa tac Glu Gly Thr Ser Gly Ser Ser Thr Val Gly Tyr Pro Gly Lys Tyr 265 270 275			929
cct tct gtc att gca gta ggc gct gtt gac agc agc aac caa aga gca Pro Ser Val Ile Ala Val Gly Ala Val Asp Ser Ser Asn Gln Arg Ala 280 285 290			977

280	285	290	
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Ser Phe Ser Ser Val Gly Pro Glu Leu Asp Val Met Ala Pro Gly Val			
295	300	305	310
tct atc caa agc acg ctt cct gga aac aaa tac ggg gcg tac aac ggt			1073
Ser Ile Gln Ser Thr Leu Pro Gly Asn Lys Tyr Gly Ala Tyr Asn Gly			
	315	320	325
acg tca atg gca tct ccg cac gtt gcc gga gcg gct gct ttg att ctt			1121
Thr Ser Met Ala Ser Pro His Val Ala Gly Ala Ala Ala Leu Ile Leu			
	330	335	340
tct aag cac ccg aac tgg aca aac act caa gtc cgc agc agt tta gaa			1169
Ser Lys His Pro Asn Trp Thr Asn Thr Gln Val Arg Ser Ser Leu Glu			
	345	350	355
aac acc act aca aaa ctt ggt gat tct ttg tac tat gga aaa ggg ctg			1217
Asn Thr Thr Thr Lys Leu Gly Asp Ser Leu Tyr Tyr Gly Lys Gly Leu			
	360	365	370
atc aac gta caa gcg gca gct cag taa a acataaaaaa ccggccttgg			1265
Ile Asn Val Gln Ala Ala Gln *			
375	380		
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gatggctccc tctgaaaatt ttaacgagaa acggcggggtt gacccggctc agtcccgtaa			1385
cggccaactc ctgaaacgtc tcaatcgccg cttcccggtt tccggtcagc tcaatgccat			1445
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Lys Ser Asn Gly Glu Lys Lys Tyr Ile Val Gly Phe Lys Gln Thr Met			
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Ser Thr Met Ser Ala Ala Lys Lys Asp Val Ile Ser Glu Lys Gly			
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Gly Lys Val Gln Lys Gln Phe Lys Tyr Val Asp Ala Ala Ser Val Thr			
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Leu Asn Glu Lys Ala Val Lys Glu Leu Lys Lys Asp Pro Ser Val Ala			
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Tyr Val Glu Glu Asp His Val Ala His Ala Tyr Ala Gln Ser Val Pro			
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Tyr Gly Val Ser Gln Ile Lys Ala Pro Ala Leu His Ser Gln Gly Tyr			
	115	120	125
Thr Gly Ser Asn Val Lys Val Ala Val Ile Asp Ser Gly Ile Asp Ser			
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Ser His Pro Asp Leu Lys Val Ala Ser Gly Ala Ser Met Val Pro Ser			
145	150	155	160
Glu Thr Asn Pro Phe Gln Asp Asn Asn Ser His Gly Thr His Val Ala			
	165	170	175
Gly Thr Val Ala Ala Leu Asn Asn Ser Ile Gly Val Leu Gly Val Ala			

Pro	Ser	Ala	180	Ser	Leu	Tyr	Ala	Val	185	Lys	Val	Leu	Gly	Ala	190	Asp	Gly	Ser
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Gly	Gln	Tyr	Ser	Trp	Ile	Ile	Asn	Gly	Ile	Glu	Trp	Ala	Ile	Ala	Asn			
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Asn	Met	Asp	Val	Ile	Asn	Met	Ser	Leu	Gly	Gly	Pro	Ser	Gly	Ser	Ala			
225				230					235						240			
Ala	Leu	Lys	Ala	Ala	Val	Asp	Lys	Ala	Val	Ala	Ser	Gly	Val	Val	Val			
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Val	Ala	Ala	Ala	Gly	Asn	Glu	Gly	Thr	Ser	Gly	Ser	Ser	Ser	Ser	Thr	Val		
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Gly	Tyr	Pro	Gly	Lys	Tyr	Pro	Ser	Val	Ile	Ala	Val	Gly	Ala	Val	Asp			
	275					280						285						
Ser	Ser	Asn	Gln	Arg	Ala	Ser	Phe	Ser	Ser	Val	Gly	Pro	Glu	Leu	Asp			
	290				295					300								
Val	Met	Ala	Pro	Gly	Val	Ser	Ile	Gln	Ser	Thr	Leu	Pro	Gly	Asn	Lys			
305				310					315					320				
Tyr	Gly	Ala	Tyr	Asn	Gly	Thr	Ser	Met	Ala	Ser	Pro	His	Val	Ala	Gly			
			325					330					335					
Ala	Ala	Ala	Leu	Ile	Leu	Ser	Lys	His	Pro	Asn	Trp	Thr	Asn	Thr	Gln			
	340						345						350					
Val	Arg	Ser	Ser	Leu	Glu	Asn	Thr	Thr	Thr	Lys	Leu	Gly	Asp	Ser	Leu			
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<211> 275

<212> PRT

<213> Bacillus amyloliquefaciens

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Ser	Gly	Ile	Asp	Ser	Ser	His	Pro	Asp	Leu	Lys	Val	Ala	Gly	Gly	Ala			
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Ser	Met	Val	Pro	Ser	Glu	Thr	Asn	Pro	Phe	Gln	Asp	Asn	Asn	Ser	His			
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Gly	Thr	His	Val	Ala	Gly	Thr	Val	Ala	Ala	Leu	Asn	Asn	Ser	Ile	Gly			
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Val	Leu	Gly	Val	Ala	Pro	Ser	Ala	Ser	Leu	Tyr	Ala	Val	Lys	Val	Leu			
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Gly	Ala	Asp	Gly	Ser	Gly	Gln	Tyr	Ser	Trp	Ile	Ile	Asn	Gly	Ile	Glu			
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Trp	Ala	Ile	Ala	Asn	Asn	Met	Asp	Val	Ile	Asn	Met	Ser	Leu	Gly	Gly			
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Pro	Ser	Gly	Ser	Ala	Ala	Leu	Lys	Ala	Ala	Val	Asp	Lys	Ala	Val	Ala			
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Ser	Gly	Val	Val	Val	Val	Ala	Ala	Ala	Gly	Asn	Glu	Gly	Thr	Ser	Gly			
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Val	Gly	Ala	Val	Asp	Ser	Ser	Asn	Gln	Arg	Ala	Ser	Phe	Ser	Ser	Val			
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Gly	Pro	Glu	Leu	Asp	Val	Met	Ala	Pro	Gly	Val	Ser	Ile	Gln	Ser	Thr			
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Leu	Pro	Gly	Asn	Lys	Tyr	Gly	Ala	Tyr	Asn	Gly	Thr	Ser	Met	Ala	Ser			
	210					215					220							

Pro His Val Ala Gly Ala Ala Ala Leu Ile Leu Ser Lys His Pro Asn  
 225 230 235 240  
 Trp Thr Asn Thr Gln Val Arg Ser Ser Leu Glu Asn Thr Thr Thr Lys  
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 Leu Gly Asp Ser Phe Tyr Tyr Gly Lys Gly Leu Ile Asn Val Gln Ala  
 260 265 270  
 Ala Ala Gln  
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 Ser Gly Ile Asp Ser Ser His Pro Asp Leu Asn Val Arg Gly Gly Ala  
 35 40 45  
 Ser Phe Val Pro Ser Glu Thr Asn Pro Tyr Gln Asp Gly Ser Ser His  
 50 55 60  
 Gly Thr His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly  
 65 70 75 80  
 Val Leu Gly Val Ser Pro Ser Ala Ser Leu Tyr Ala Val Lys Val Leu  
 85 90 95  
 Asp Ser Thr Gly Ser Gly Gln Tyr Ser Trp Ile Ile Asn Gly Ile Glu  
 100 105 110  
 Trp Ala Ile Ser Asn Asn Met Asp Val Ile Asn Met Ser Leu Gly Gly  
 115 120 125  
 Pro Thr Gly Ser Thr Ala Leu Lys Thr Val Val Asp Lys Ala Val Ser  
 130 135 140  
 Ser Gly Ile Val Val Ala Ala Ala Gly Asn Glu Gly Ser Ser Gly  
 145 150 155 160  
 Ser Thr Ser Thr Val Gly Tyr Pro Ala Lys Tyr Pro Ser Thr Ile Ala  
 165 170 175  
 Val Gly Ala Val Asn Ser Ser Asn Gln Arg Ala Ser Phe Ser Ser Ala  
 180 185 190  
 Gly Ser Glu Leu Asp Val Met Ala Pro Gly Val Ser Ile Gln Ser Thr  
 195 200 205  
 Leu Pro Gly Gly Thr Tyr Gly Ala Tyr Asn Gly Thr Ser Met Ala Thr  
 210 215 220  
 Pro His Val Ala Gly Ala Ala Ala Leu Ile Leu Ser Lys His Pro Thr  
 225 230 235 240  
 Trp Thr Asn Ala Gln Val Arg Asp Arg Leu Glu Ser Thr Ala Thr Tyr  
 245 250 255  
 Leu Gly Asn Ser Phe Tyr Tyr Gly Lys Gly Leu Ile Asn Val Gln Ala  
 260 265 270  
 Ala Ala Gln  
 275

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 <212> PRT  
 <213> Bacillus licheniformis

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Thr	Gly	Ile	Gln	Ala	Ser	His	Pro	Asp	Leu	Asn	Val	Val	Gly	Gly	Ala
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Ser	Phe	Val	Ala	Gly	Glu	Ala	Tyr	Asn	Thr	Asp	Gly	Asn	Gly	His	Gly
	50					55					60				
Thr	His	Val	Ala	Gly	Thr	Val	Ala	Ala	Leu	Asp	Asn	Thr	Thr	Gly	Val
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Leu	Gly	Val	Ala	Pro	Ser	Val	Ser	Leu	Tyr	Ala	Val	Lys	Val	Leu	Asn
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Ser	Ser	Gly	Ser	Gly	Ser	Tyr	Ser	Gly	Ile	Val	Ser	Gly	Ile	Glu	Trp
		100						105					110		
Ala	Thr	Thr	Asn	Gly	Met	Asp	Val	Ile	Asn	Met	Ser	Leu	Gly	Gly	Ala
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Ser	Gly	Ser	Thr	Ala	Met	Lys	Gln	Ala	Val	Asp	Asn	Ala	Tyr	Ala	Arg
	130					135					140				
Gly	Val	Val	Val	Val	Ala	Ala	Ala	Gly	Asn	Ser	Gly	Asn	Ser	Gly	Ser
145					150					155					160
Thr	Asn	Thr	Ile	Gly	Tyr	Pro	Ala	Lys	Tyr	Asp	Ser	Val	Ile	Ala	Val
				165					170					175	
Gly	Ala	Val	Asp	Ser	Asn	Ser	Asn	Arg	Ala	Ser	Phe	Ser	Ser	Val	Gly
			180					185					190		
Ala	Glu	Leu	Glu	Val	Met	Ala	Pro	Gly	Ala	Gly	Val	Tyr	Ser	Thr	Tyr
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Pro	Thr	Asn	Thr	Tyr	Ala	Thr	Leu	Asn	Gly	Thr	Ser	Met	Ala	Ser	Pro
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His	Val	Ala	Gly	Ala	Ala	Ala	Leu	Ile	Leu	Ser	Lys	His	Pro	Asn	Leu
225				230						235					240
Ser	Ala	Ser	Gln	Val	Arg	Asn	Arg	Leu	Ser	Ser	Thr	Ala	Thr	Tyr	Leu
				245					250					255	
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Ala Gln

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 <211> 269  
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Thr	Gly	Ile	Ser	Thr	His	Pro	Asp	Leu	Asn	Ile	Arg	Gly	Gly	Ala	Ser
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Phe	Val	Pro	Gly	Glu	Pro	Ser	Thr	Gln	Asp	Gly	Asn	Gly	His	Gly	Thr
	50					55					60				
His	Val	Ala	Gly	Thr	Ile	Ala	Ala	Leu	Asn	Asn	Ser	Ile	Gly	Val	Leu
65					70					75					80
Gly	Val	Ala	Pro	Ser	Ala	Glu	Leu	Tyr	Ala	Val	Lys	Val	Leu	Gly	Ala
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Ser	Gly	Ser	Gly	Ser	Val	Ser	Ser	Ile	Ala	Gln	Gly	Leu	Glu	Trp	Ala
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Gly	Asn	Asn	Gly	Met	His	Val	Ala	Asn	Leu	Ser	Leu	Gly	Ser	Pro	Ser
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Pro	Ser	Ala	Thr	Leu	Glu	Gln	Ala	Val	Asn	Ser	Ala	Thr	Ser	Arg	Gly
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Val	Leu	Val	Val	Ala	Ala	Ser	Gly	Asn	Ser	Gly	Ala	Gly	Ser	Ile	Ser
145					150					155					160

Tyr	Pro	Ala	Arg	Tyr	Ala	Asn	Ala	Met	Ala	Val	Gly	Ala	Thr	Asp	Gln
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Asn	Asn	Asn	Arg	Ala	Ser	Phe	Ser	Gln	Tyr	Gly	Ala	Gly	Leu	Asp	Ile
			180					185					190		
Val	Ala	Pro	Gly	Val	Asn	Val	Gln	Ser	Thr	Tyr	Pro	Gly	Ser	Thr	Tyr
		195					200					205			
Ala	Ser	Leu	Asn	Gly	Thr	Ser	Met	Ala	Thr	Pro	His	Val	Ala	Gly	Ala
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Ala	Ala	Leu	Val	Lys	Gln	Lys	Asn	Pro	Ser	Trp	Ser	Asn	Val	Gln	Ile
225					230					235					240
Arg	Asn	His	Leu	Lys	Asn	Thr	Ala	Thr	Ser	Leu	Gly	Ser	Thr	Asn	Leu
				245					250					255	
Tyr	Gly	Ser	Gly	Leu	Val	Asn	Ala	Glu	Ala	Ala	Thr	Arg			
			260					265							